RELATIONSHIPS BETWEEN CELL POPULATION KINETICS AND RADIATION RESISTANCE IN POCKET MICE (HETEROMYIDAE: PEROGNATHUS)

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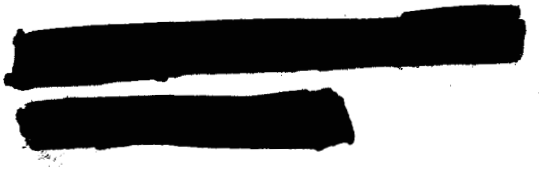
To be presented to:

Third International Congress
of Radiation Research

To be held at Cortina, Italy
June 26 - July 2, 1966

FACILITY FORM 602

N68-18954 (ACCESSION NUMBER) — (THRU)
10 (PAGES) — 1 (CODE)
NASA-CR-69454 (NASA CR OR TMX OR AD NUMBER) — 04 (CATEGORY)



ABSTRACT

Pocket mice (Perognathus longimembris), subjected to 1000 and 1500 rad whole body Co^{60} irradiation, were sacrificed in groups of 5 on days 1, 2, 5, 6, 7 post-irradiation. Degenerative changes were noted in the intestinal mucosa within hours after irradiation, but visible damage was confined mainly to intestinal crypts. Villi remained intact throughout the observation period, but showed some reduction in size. Regeneration of mucosal epithelium was prompt, restoring villi to normal by the 7th day even after 1500 rad. Tritiated thymidine studies indicate that intestinal epithelium cells of pocket mice have a villus transit time of 5.7 days. This slow rate of villus attrition probably enhances post-irradiation survival, accounting, in part, for the high $LD_{50/30}$ (1500 r) reported for this species.

INTESTINAL MUCOSA DYNAMICS OF THE LITTLE POCKET MOUSE (PEROGNATHUS LONGIMEMBRIS)

J. J. Gambino and J. W. Towner

Introduction

Earlier we suggested that pocket mouse radiation resistance might be due, in part, to a slow rate of loss of epithelial cells from the intestinal villi (3). One consequence of a slow sloughing rate would be a slower rate of villus attrition and, effectively, protection from the gastrointestinal syndrome. This suggestion was predicated on the fact that no deaths occur in pocket mice during the first week after high dose whole body irradiation, a time when conventional mice and rats succumb to the gastrointestinal syndrome. Furthermore, dose survival time curves of pocket mice and germfree mice show remarkable similarities, with the plateau representing gastrointestinal death displaced upward and shortened for both species (1,5,6). Since this shift of the plateau was attributed to certain peculiarities in cell population dynamics of the intestinal mucosa of germfree mice, similar mechanisms were sought in pocket mice.

In two experiments that are reported herein, early post-irradiation histopathology of the intestine was examined, and the normal transit-time of pocket mouse intestinal epithelial cells was determined.

Methods and Materials

Field trapped, adult Perognathus longimembris, which had been maintained in our laboratory for approximately one year were used. Environmental conditions and animal husbandry are described elsewhere (2,3). Ambient temperature and humidity were maintained at 22° $\pm$ 2°C and 50 $\pm$ 5%, respectively. The animals were individually housed and fed a mixture of grass seeds and sunflower seeds. Since pocket mice have no requirement for drinking water, no water was provided. Lights were turned on at 0630 hours and off at 0530 hours PST.

Twenty-five male pocket mice were used in the first experiment. They were divided into 3 groups: two groups were given a single, acute whole body dose of either 1000 rad or 1500 rad, and one was kept as control.

Irradiation was delivered from a 5000 curie Co^{60} source at 24.5 rad/min for the 1000 rad dose, and at 37.8 rad/min for the 1500 rad dose. Ferric sulphate and phosphate glass dosimetry was used.

Animals were sacrificed at selected intervals up to one week post-irradiation. Sections of small intestine were removed and fixed in 10% formalin, sectioned at 7μ and stained with haematoxylin-eosin.

For the second experiment 20 P. longimembris of both sexes were randomly divided into 5 groups of 4 mice each. All 20 animals were administered 10μc of tritiated thymidine (Schwarz Bioresearch, Inc. Lot #1402, Sp. Ac. 0.36c/mM) in 0.1 ml physiological saline intraperitoneally at the start of the experiment.

At 12, 24, 72, 120 and 168 hours post-injection, four animals were sacrificed by ether anaesthesia and a section of jejunum was fixed in Bouin's.

Autoradiographs were prepared in the usual manner with Kodak NTB-2 nuclear track emulsion*. Some were developed after 10 days and some after 30 days exposure. Haematoxylin-eosin stain was used after development.

Two slides were prepared for each animal, providing ample material for scanning. The percentage of villus height traversed by labeled cells was judged by scanning all the sections prepared for each animal and measuring the distance traveled by the most advanced labeled cell front. This "front" appeared to be sufficiently representative of all the labeled cells on any particular slide.

Results

The intestinal mucosa of pocket mice sacrificed at 6 and 15 hours after receiving 1000 rad and 1500 rad total body gamma irradiation showed the usual degenerative changes of irradiation damage. Villi remained intact but the villus epithelial cells showed increased cytoplasmic vacuolation and some distortion in size and shape. Nuclei of villus epithelial cells remained relatively uniform in size and staining qualities. Cells of the crypts of Lieberkühn showed much greater changes than those of the villi. Their cytoplasm was highly vacuolated and considerable nuclear damage was evident. Chromatin debris and cellular fragments predominated in the crypts. Few mitotic figures could be seen, and those that were visible appeared abnormal.

*Appreciation is expressed to Dr. A. C. Upton and William D. Gude of Oak Ridge National Laboratory for the preparation of these autoradiographs.

At 30 to 39 hours post-irradiation the villi were still intact, although they appeared to be slightly shortened. Nuclear and other cellular debris was cleared from most of the crypts, and a few mitotic figures were observed.

By the 4th day post-irradiation, crypts appeared normal except for an increased rate of mitosis as compared with controls.

Between the 5th and 7th days, movement of regenerated epithelial cells up the villus was well marked by the characteristic basophilic staining of new cells. It was at this time that differences in the 1000 rad and 1500 rad groups were seen. By day 6 the replacement of villus epithelium was completed in the 1000 rad animals, while the 1500 rad animals showed the regeneration wave only part way up the villus. It was difficult to differentiate between control slides and slides of either of the irradiated groups on day 7.

During the course of the post-irradiation period, neither irradiated group showed appreciable degeneration of the villus. At these dose levels villus epithelial cells tended to remain intact on the villus core until replaced by regenerated cells.

Figure 1 shows the percentage of villus height traversed by labeled cells at intervals in the 7 days following injection with tritiated thymidine.

Although the data were not obtained in precisely the same manner, Figure 2 compares transit times of pocket mice, germfree mice and conventional mice (5). The points plotted in Figure 2 for the pocket mouse were obtained by averaging the distances represented by bars in Figure 1. These data demonstrate a longer villus transit time for pocket mouse epithelial cells, than for either germfree or conventional mice. The estimated times for reaching the villus tip are 2.1 days for conventional mice, 4.3 days for germfree mice, and 5.7 for pocket mice.

Discussion

Damage to the gastrointestinal mucosa is responsible in a large measure for early deaths in mammals after whole body radiation doses ranging between 1000 and 10,000 rad. In this dose range, death is generally attributed to denudation of the intestinal mucosa with concomitant bloody diarrhea, electrolyte loss, bacterial invasion of tissues, etc., which are all manifestations of the acute gastrointestinal syndrome. Germfree mice and

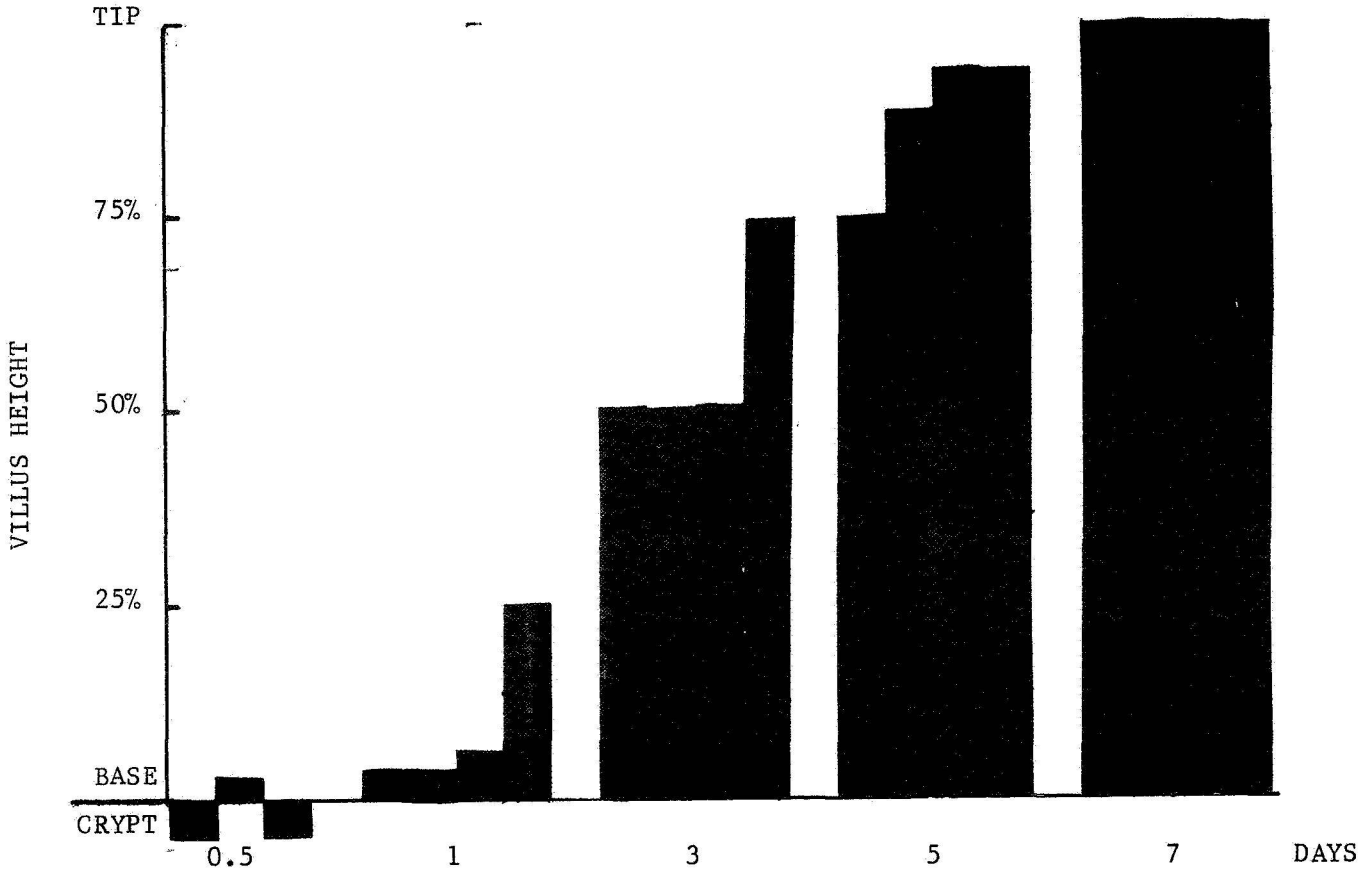


FIGURE 1 - Progress of thymidine-H^3-labeled cells on villi of pocket mouse jejunum expressed as percentage of villus height traversed by the labeled cell "front". Each bar represents one animal.

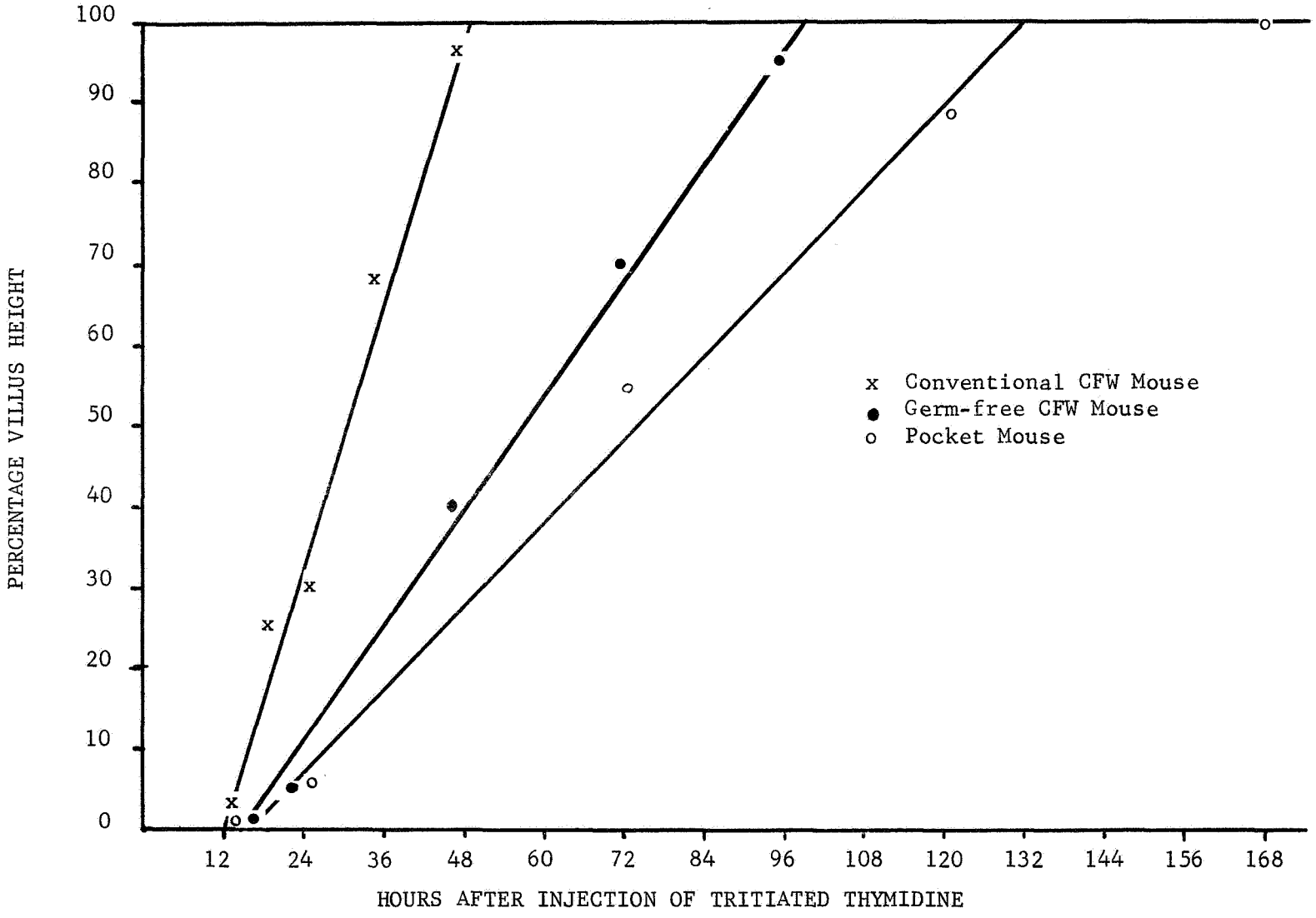


FIGURE 2 - Progress of thymidine-H^3-labeled cells on villi of pocket mice compared with values for germfree and conventional mice reported by Matsuzawa and Wilson (Rad. Res. 25, 15-24, 1965)

several species of wild rodents, which show various degrees of radiation resistance, appear to bypass the acute gastrointestinal syndrome (2,4,5,6). It has been suggested for germfree mice that survival is the result of a much slower rate of villus cell loss. This slow loss of epithelial cells tends to maintain the villi intact after high dose irradiation, thereby enhancing survival. For example, conventional mice and rats, if given supralethal doses, die at about 2 to 4 days post-irradiation. The 2 to 4 day time period is about twice as long as villus transit time, which in turn is a reliable measure of the rate of cell loss at the villus tip. Germfree mice, on the other hand, have a longer survival time after equivalent doses, generally surviving for 7 days (5,6). This survival time, too, is approximately double the villus transit time for germfree mice.

Since germfree mice have a slower rate of sloughing at the villus tip than do conventional mice, the entire cell renewal system involved in replenishing the gut mucosa is slowed. Therefore, other factors, such as decreased mitotic activity, and decreased cellular activity, as well as the absence of bacteria all operate to produce the net effect in germfree mice.

Pocket mice, which survive 8 to 12 days following supralethal irradiation, have now been shown to have an even longer transit time than germfree mice. Since transit time is considered a measure of sloughing rate, much of the radiation resistance of pocket mice can be attributed to the fact that the integrity of the gastrointestinal epithelium is maintained even after high dose irradiation. The histological picture of pocket mouse intestine after 1500 rad irradiation tends to corroborate this point.

Summary

Pocket mice subjected to 1000 rad and 1500 rad whole body Co^{60} irradiation were sacrificed in the one week period following exposure. Histopathology of the intestinal mucosa was examined. Degenerative changes were noted within hours after irradiation, but damage was confined mainly to intestinal crypts, while villi remained intact throughout the period of observation. Regeneration of epithelial cells was prompt, and even at the 1500 rad dose level, was completed by about 7 days post-irradiation.

Tritiated thymidine studies indicate that pocket mice have a villus transit time of 5.7 days for intestinal mucosa cells in contrast to 4.3 days and 2.1 days for germfree and conventional CFW mice, respectively. The differences between germfree and conventional CFW mice and the marked contrast between CFW mice and pocket mice in response to a given dose of radiation may reflect, in part, these differences in villus transit time.

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